AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of all claims in the application.

Listing of Claims

1. **(Currently amended)** A method of identifying a ~~transmembrane receptor (TMR)~~ **G protein coupled receptor (GPCR)** agonist, wherein the ~~TMR agonist (TMRA)~~ **GPCR agonist** is capable of activating ~~TMR~~ **GPCR** signaling while exhibiting reduced ~~TMR~~ **GPCR** internalization ~~over~~ **as compared to** a control compound, comprising the steps of:

(a) providing a cell comprising at least one **GPCR or a modified GPCR capable of activating intracellular signaling** ~~TMR, or a biologically active fragment thereof, wherein the cell further comprises~~ **and an** arrestin, or a ~~biologically active~~ fragment thereof **capable of binding a GPCR**,

(b) exposing the cell to at least one test compound,

(c) measuring [[the]] **GPCR** signaling at two or more points in time,

(d) measuring ~~the translocation~~ **internalization** of the ~~TMR~~ **GPCR** at two or more points in time,

(e) quantitatively determining if ~~the TMR~~ **GPCR** internalization is reduced by comparing ~~the TMR~~ **GPCR** internalization in the presence of the test compound to ~~the TMR~~ **GPCR** internalization in the presence of a control compound, and wherein ~~the~~ **GPCR** signaling is activated **in the presence of the test compound** as compared to ~~TMR~~ **GPCR** signaling in the absence of **the test compound,** ~~agonist; and~~

(f) wherein ~~reduced TMR~~ **a reduction in GPCR** internalization **in the presence of the test compound as compared to the control compound indicates the test compound is a GPCR** ~~identifies a TMR~~ agonist **capable of activating GPCR signaling while exhibiting reduced GPCR internalization**.

2. **(Canceled)** ~~The method of claim 1, wherein the TMR is a GPCR.~~

3. **(Currently Amended)** The method of claim 1, wherein the ~~translocation~~ **internalization** of the ~~TMR~~ **GPCR** is measured by monitoring localization of a detectable molecule bound to the arrestin or **to** the ~~TMR~~ **GPCR**.

4. **(Previously Presented)** The method of claim 1, wherein signaling is quantitated by measuring an intracellular effector, wherein said effector is cAMP, cyclic GMP, calcium, a lipid, phosphatidylinositol, a hydrogen ion, or an ion transport molecule.

5-7. **(Canceled)**

8. **(Currently Amended)** The method of claim 1, wherein the signaling is activated for a longer time period after stimulation by the ~~TMRA~~ **test compound** than the length of time of activation after stimulation by the control compound.

9. **(Currently Amended)** The method of claim 1, wherein the ~~translocation~~ **internalization** of the ~~TMR~~ **GPCR** is measured by determining the localization **of the GPCR** in the plasma membrane, pits, endosomes, endocytic vesicles, or cytosol.

10. **(Currently Amended)** The method of ~~claim 2~~ **claim 1**, wherein the GPCR is a class A, or class B receptor.

11. **(Currently Amended)** The method of ~~claim 2~~ **claim 1**, wherein the GPCR is a μ opioid, β_1AR, β_2AR, or dopamine receptor.

12. **(Currently Amended)** The method of claim 1, wherein the ~~translocation~~ **internalization** of the ~~TMR~~ **GPCR** is measured by visualization of a radioisotope, an epitope tag, an affinity label, an enzyme, a fluorescent group, or a chemiluminescent group attached to the arrestin or the ~~TMR~~ **GPCR**.

13. **(Currently amended)** The method of claim 1, wherein the signaling is measured at the same time as the ~~translocation~~ **internalization** is measured.

14. **(Original)** The method of claim 1, wherein the cell is exposed to the compound once, and wherein the cell is not exposed a second time to the compound.

15. **(Withdrawn)** A compound discovered by the method of claim 1.

16. **(Currently Amended)** The method of claim 1, wherein the ~~TMR~~ **GPCR** is a rat, mouse, pig, or primate ~~TMR~~ **GPCR**.

17. **(Currently Amended)** The method of claim 1, wherein the ~~method is~~ **steps (a) – (f) are** repeated, and wherein the ~~TMR~~ **GPCR used** in the **repeated steps** ~~repeat method~~ is from a different species than **the GPCR used** in ~~the original method~~ **steps (a) – (f)**.

18. **(Currently Amended)** The method of claim 17, wherein a test compound that is **used in steps (a) – (f)** ~~a TMRA in the original method~~ is not **used** ~~a TMRA~~ in the **repeated steps** ~~repeat method, and wherein the repeat method contains a TMR from a different species~~.

19. **(Previously Presented)** The method of claim 1, wherein the test compound is from a combinatorial library.

20. **(Previously Presented)** The method of claim 1, wherein the signaling in the presence of the test compound is equal to or greater than the signaling in the presence of the control compound.

21. **(Currently amended)** The method of claim 1, wherein the method is repeated at different concentrations of compound to yield a ~~dose response~~ **dose-response** curve for the signaling measurement and a ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the test compound.

22. **(Currently amended)** The method of claim 21, wherein the quantitative determination includes a comparison of the ~~dose response~~ **dose-response** curve for the signaling measurement to the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement.

23. **(Currently amended)** The method of claim 21, wherein a second ~~dose response~~ **dose-response** curve for the signaling measurement and a second ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement are determined in the presence of control compound.

24. **(Currently amended)** The method of claim 23, wherein ~~dose response~~ **the dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the test compound is ~~reduced as compared to the dose response~~ **less than the dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the control compound.

25. **(Currently amended)** The method of claim 23, wherein the ~~dose response~~ **dose-response** curve for the signaling measurement in the presence of the test compound is approximately equal to or greater than the ~~dose response~~ **dose-response** curve for the signaling measurement in the presence of the control compound.

26. **(Currently amended)** The method of claim 24, wherein the reduced ~~translocation~~ **internalization** is determined by a decrease in the Max of the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the test compound, as compared to

the Max of the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the control compound.

27. **(Currently amended)** The method of claim 24, wherein the reduced ~~translocation~~ **internalization** is determined by an increase in the EC50 of the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the test compound, as compared to the EC50 of the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the control compound.

28-54. **(Canceled)**

55. **(Currently Amended)** A method of identifying a ~~transmembrane receptor (TMR)~~ **G protein coupled receptor (GPCR)** agonist, wherein the **GPCR agonist** ~~TMR agonist (TMRA)~~ is capable of activating TMR signaling while exhibiting reduced TMR internalization over a control compound, comprising the steps of:

(a) providing a cell comprising at least one **GPCR or a modified GPCR capable of activating intracellular signaling** ~~TMR, or a biologically active fragment thereof, wherein the cell further comprises~~ **and an** arrestin, or a ~~biologically active~~ fragment thereof **capable of binding a GPCR**,

(b) exposing the cell to at least one test compound,

(c) measuring [[the]] **GPCR** signaling at one or more concentration of **the test** compound,

(d) measuring ~~the translocation~~ **internalization** of the ~~TMR~~ **GPCR** at one or more concentration of **the test** compound,

(e) quantitatively determining if ~~the TMR~~ **GPCR** internalization is reduced by comparing ~~the TMR~~ **GPCR** internalization in the presence of the test compound to ~~the TMR~~ **GPCR** internalization in the presence of a control compound, and wherein ~~the~~ **GPCR** signaling is activated **in the presence of the test compound** as compared to ~~TMR~~ **GPCR** signaling in the absence of **the test compound,** ~~agonist; and~~

(f) wherein ~~reduced TMR~~ **a reduction in GPCR** internalization **in the presence of the test compound as compared to the control compound indicates the test compound is a GPCR**

~~identifies a TMR~~ agonist **capable of activating GPCR signaling while exhibiting reduced GPCR internalization**.

56. **(Canceled)** ~~The method of claim 55, wherein the TMR is a GPCR.~~

57. **(Currently Amended)** The method of claim 55, wherein the ~~translocation~~ **internalization** of the ~~TMR~~ **GPCR** is measured by monitoring localization of a detectable molecule bound to the arrestin or **to** the ~~TMR~~ **GPCR**.

58. **(Previously Presented)** The method of claim 55, wherein signaling is quantitated by measuring an intracellular effector, wherein said effector may be cAMP, cyclic GMP, calcium, a lipid, phosphatidylinositol, a hydrogen ion, or an ion transport molecule.

59 –61. **(Cancelled).**

62. **(Currently Amended)** The method of claim 55, wherein the signaling is activated for a longer time period after stimulation by the ~~TMRA~~ **test compound** than the length of time of activation after stimulation by the control compound.

63. **(Currently Amended)** The method of claim 55, wherein the ~~translocation~~ **internalization** of the ~~TMR~~ **GPCR** is measured by determining the localization **of the GPCR** in the plasma membrane, pits, endosomes, endocytic vesicles, or cytosol.

64. **(Currently Amended)** The method of ~~claim 56~~ **claim 55**, wherein the GPCR is a class A, or class B receptor.

65. **(Currently Amended)** The method of ~~claim 56~~ **claim 55**, wherein the GPCR is a μ opioid, β_1AR, β_2AR, or dopamine receptor.

66. **(Currently Amended)** The method of claim 55, wherein the ~~translocation~~ **internalization** of the ~~TMR~~ **GPCR** is measured by visualization of a radioisotope, an epitope tag, an affinity label, an enzyme, a fluorescent group, or a chemiluminescent group attached to the arrestin or the ~~TMR~~ **GPCR**.

67. **(Currently amended)** The method of claim 55, wherein the signaling is measured at the same time as the ~~translocation~~ **internalization** is measured.

68. **(Original)** The method of claim 55, wherein the cell is exposed to the compound once, and wherein the cell is not exposed a second time to the compound.

69. **(Withdrawn)** A compound discovered by the method of claim 55.

70. **(Currently Amended)** The method of claim 55, wherein the ~~TMR~~ **GPCR** is a rat, mouse, pig, or primate ~~TMR~~ **GPCR**.

71. **(Currently Amended)** The method of claim 55, wherein the ~~method is~~ **steps (a) – (f) are** repeated, and wherein the ~~TMR~~ **GPCR used** in the **repeated steps** ~~repeat method~~ is from a different species than **the GPCR used** in ~~the original method~~ **steps (a) – (f)**.

72. **(Currently Amended)** The method of claim 71, wherein a test compound that is **used in steps (a) – (f)** ~~a TMRA in the original method~~ is not **used** a TMRA in the **repeated steps** ~~repeat method, and wherein the repeat method contains a TMR from a different species~~.

73. **(Previously Presented)** The method of claim 55, wherein the test compound is from a combinatorial library.

74. **(Original)** The method of claim 55, wherein the signaling in the presence of the test compound is approximately equal to or greater than the signaling in the presence of the control compound.

75. **(Currently amended)** The method of claim 55, wherein the method is repeated at different concentrations of compound to yield a ~~dose response~~ **dose-response** curve for the signaling measurement and a ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the test compound.

76. **(Currently amended)** The method of claim 75, wherein the quantitative determination includes a comparison of the ~~dose response~~ **dose-response** curve for the signaling measurement to the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement.

77. **(Currently amended)** The method of claim 75, wherein a second ~~dose response~~ **dose-response** curve for the signaling measurement and a second ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement are determined in the presence of control compound.

78. **(Currently amended)** The method of claim 77, wherein ~~dose response~~ **the dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the test compound is ~~reduced as compared to the dose response~~ **less than the dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of, the control compound.

79. **(Currently amended)** The method of claim 77, wherein the ~~dose response~~ **dose-response** curve for the signaling measurement in the presence of the test compound is approximately equal to or greater than the ~~dose response~~ **dose-response** curve for the signaling measurement in the presence of the control compound.

80. **(Currently amended)** The method of claim 78, wherein the reduced ~~translocation~~ **internalization** is determined by a decrease in the Max of the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the test compound, as compared to the Max of the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the control compound.

81. **(Currently amended)** The method of claim 78, wherein the reduced ~~translocation~~ **internalization** is determined by an increase in the EC50 of the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the test compound, as compared to the EC50 of the ~~dose response~~ **dose-response** curve for the ~~translocation~~ **internalization** measurement in the presence of the control compound.

82-108 **(Canceled)**

109. **(Currently Amended)** The method of claim 16, wherein the primate ~~TMR~~ **GPCR** is a human ~~TMR~~ **GPCR**.

110. **(Currently Amended)** The method of claim 70, wherein the primate ~~TMR~~ **GPCR** is a human ~~TMR~~ **GPCR**.